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BIOLOGICAL BULLETIN

THE PROTOZOAN LIFE CYCLE.¹

GARY N. CALKINS.

Twenty years ago it was an amusing pastime to see with the aid of the microscope, and to describe, new and interesting forms of unicellular organisms. Today there is not a field of biological science that is not illumined by the deeper study of the protozoa, and the pastime of our fathers has become the science of protozoology to-day. In its modern aspect this new science has many sides, morphology, physiology, psychology, cytology, and pathology, and although there is little danger of its being cut up into unrecognizable parts, there is need of some ground principle or principles to hold the many branches of protozoa study together and to unify the science. It was the genius of Schaudinn, whose untimely death has taken from protozoölogy its most brilliant light, to establish such an unifying foundation, and in his clear perception of the importance of the life cycle we have the key note of our present day conception of the protozoa. With our present knowledge we may define protozoa as: Independent, unicellular, animal organisms which reproduce by division or spore formation, the progeny passing through various phases of activity collectively known as the life cycle, and manifesting various degrees of vitality with accompanying form changes.

In thus emphasizing the life cycle in the definition I would seek to introduce into protozoa study the recognition of the entire cycle of changes as a necessary basis for species. I would have the presentation of the life history of a protozoön a prerequisite for its acceptance as a new species and would have zoölogists recog-

¹ Substance of addresses given before Sections D and K of the British Association for the Advancement of Science, at York, 1906.

nize that a new species can no more be created on the basis of a single cell or group of cells in the same stage of the life cycle than a species of mammal can be established on the basis of a fore limb, a jaw bone, or a tooth. It seems to me high time that the science of protozoa-study should be freed from the charge of dilettantism, and time for the literature to be cleared of the great burden of synonyms that must ever increase so long as novices in this field of study see and describe in print what to them are new and curious forms.

There is enough known at the present time of protozoan life cycles to indicate that for a given species the cycle under similar conditions is always the same, and we are justified in considering the entire congeries of forms which the protozoön passes through in its life cycle, and not the single cell, as the "individual," comparable indeed, as has long since been pointed out, to the metazoön. There is nothing fanciful in comparing the rapid asexual phase of a protozoön with the proliferation of somatic cells of a metazoön, or the periods of conjugation and old age in a life cycle with the sexual maturity and senescence in metazoa. So many different forms are assumed by the protozoön in the numerous stages of vitality, that, unless the entire cycle is known, even the skilled observer might be justified in considering the various phases of the same organism as different organisms. Instances of this confusion come to your minds at once, and I need but mention Plasmodium and Polymitus, Coccidium and Eimeria to illustrate my meaning. The same confusion has recently come under my observation in connection with *Paramecium aurelia* and *P. caudatum*, which, since the classical work of Maupas, have been generally but erroneously accepted as distinct species.

The specific differences between these two supposedly different forms have been emphasized by Maupas and Hertwig, and more recently by Simpson, and are based upon some minor characteristics of size and form but mainly upon the presence of two micronuclei in *P. aurelia* and one in *P. caudatum*. In all cultures of *P. caudatum*, epidemics of conjugation appear at more or less regular intervals. Four pairs of conjugating forms were isolated from such a culture on March 11, 1905, and after separation the eight individuals were isolated and their several histories followed

out until the race in each case became extinct. Two of the eight individuals representing different pairs continued to multiply in cultures for more than a year, the method employed being the same as that used in previous experiments.¹ The other six died before reaching the fourth generation. One of the two successful exconjugants reorganized as *Paramecium aurelia*, the others as *P. caudatum*. The *caudatum* form was much more vigorous than the *aurelia* form and kept up in its division rate with a ninth individual that was chosen for control from the individuals that had not conjugated in the original culture. At the end of two months the *aurelia* form had divided 55 times, the *caudatum* form 76 and the control 77 times. In the period between the last of April and the end of June the *aurelia* form gradually lost its specific *aurelia* characters and became more and more like *caudatum*. The two micronuclei were reduced to one, one being absorbed, and by the seventieth generation the original *aurelia* form could not be distinguished morphologically from the original *caudatum* form, while its division energy became much greater than that of the other types in culture, an energy which lasted through more than 300 generations.

Without entering into a discussion of the interesting biological features of this apparent change of species which I have published elsewhere,² I would merely call attention to the fact that here at any time, during the first 45 generations at least, the thousands of cells that might have been formed would have been classified as *P. aurelia*, while study of the life history shows that it is only a temporary form assumed by *P. caudatum* and is to be interpreted as a mere variant or sport. These results merely emphasize the importance of studying the entire life cycle as a basis for protozoan species.

It is particularly important that these specific distinctions should be clearly recognized in that field of protozoan research which is most important at the present time, — the pathogenic. Here more than in any other field of biological study is the need

¹ Calkins, "Studies on the Life History of Protozoa," I., *Arch. f. Entw.*, XV., No. 1, 1902.

² *Paramecium aurelia* and *Paramecium caudatum*, in "Biological Studies" by the pupils of William Thompson Sedgwick, Chicago, 1906.

of a clear conception of the different phases of the parasite, and the possibility of different hosts or of different effects on the same hosts at different periods of vitality should be known to the pathologists. The tendency to put protozoa on the same basis of research as the bacteria, despite the brilliant work in cultivating certain types of protozoa on artificial media, which one of my countrymen regards as the *sine qua non* of pathogenic protozoan research, seems to me to be a step in the wrong direction. To study parasitic protozoa in culture is to study them under one condition only, and in one phase only of the life history, and the different forms that are met with in such artificial media may be more often involution types than normal phases, and the great multiplication of species of *Trypanosoma* or *Spirochaeta* bespeaks perhaps more than any other one thing the presence of a new type of novitiate in protozoan research.

If the life cycle is to be accepted as the basis of new species, and regarded as the individual in a taxonomic sense, it should be sufficiently definite to be taken as a unit, and the life histories of widely separated species should have some common grounds for comparison. Thanks to the great stimulus given in recent years to protozoan study, we know the full life history of many widely separated forms, and at the present time we are able to generalize to some extent and to formulate a few ground principles. The old-time comparison of the metazoön with the mass of cells that are formed by the repeated division of the first parent cell of a protozoön after conjugation, can be amplified and extended at the present time to the comparison with the metazoön of not only the mass or morphology, but the physiology or general biology of the constituent cells as well. As with the metazoön so with the aggregate of protozoa cells, we note a period of youth characterized by active cell-proliferation; this in both groups of organisms is followed by the gradual loss of the division energy accompanied by morphological changes in type of the cells preliminary to conjugation and fertilization and to the renewal of vitality by this means. When such renewal is omitted, and for one reason or another this stage is never reached by the great majority of protozoa, the third characteristic period — old age — supervenes and the race of pro-

tozoa dies out from protoplasmic senility no less surely than does the body of the metazoön. We can distinguish, then, in the life history of a protozoön three more or less clearly marked stages, youth, adolescence, and old age, each with certain characteristics but which we cannot sharply mark off one from another any more than we can clearly limit the three stages of a metazoön.

As with the fertilized egg of a metazoön, the copula or fertilized cell of a protozoön is endowed with a great power of cell-reproduction and with a high potential of vitality, and this is the main characteristic of the first period of the life cycle. This reproduction may take the form of simple division, of budding, or of spore formation, according to the difficulties that have been successfully overcome by the species in its struggle for existence. The young forms show well marked conformity to type, and this feature, occurring when the greatest numbers of representatives of the species are in evidence, undoubtedly has given a false impression of the stability of form of the protozoan species.

This is the period also of the greatest resistance to adverse conditions of the surrounding medium and in pathogenic forms it is the period of greatest malignancy. It is a well-known fact that, in many parasitic forms of protozoa, attempts to inoculate from animal to animal are either failures altogether or result in a weakened race of the organisms; these failures are perhaps due to the inability of the organisms in a more or less weakened condition to withstand the natural immunity of the host, which they are perfectly able to do with the full potential of vitality with which they are endowed after conjugation. In some cases, as for example in *Trypanosoma*, the natural vitality of the parasite is so much greater than the natural resistance of the host that such inoculation is possible and the transplanted organisms continue to live. The matter of malignancy is so intimately connected with this question of restored vitality that, in yellow fever for example, it alone is almost sufficient to indicate that conjugation processes must take place in the body of *Stegomyia fasciata*.

This first period is then marked by a distinct excess of constructive over destructive metabolism and in the series of divisions or repeated spore formation which follows fertilization there is a

gradual tendency for the energy of multiplication to weaken, or, as we may express it, the constructive and destructive processes tend to equalize. With the decline of the division rate comes the advent of the second period in which the most important functions in the life of the protozoön occur.

The main characteristic of this second general period, or adolescence, is a general decline in the multiplication rate and more or less definite change in form of the cells and in their chemical and physical composition. A single cell, unless it is in the sexual phase, gives little or no clue to its stage in the life history. In the majority of cases it is only by the study of a long series that the student is able to recognize this period in the life cycle. In such a study, which one can easily carry on with certain free forms of ciliates, the decline of the division rate with advancing age of the series is easily followed. In a form like *Paramecium*, for example, where there is no sexual dimorphism, one notes at this period a change in the physical constitution of the protoplasm. Such changes, leading in *Paramecium* to what I have termed the "miscible" state, are certainly the most striking features in the life history of protozoa and *a fortiori* of the period of adolescence, for in them we find an explanation of all the form changes that take place in any life history. Such form changes may involve only the cytoplasm as in *Paramecium* or *Tetramitus*, only the nucleus as in *chromidium* formation in rhizopods, or both nucleus and cytoplasm as in gamete formation with or without sexual differentiation.

In *Paramecium* this condition comes at a period which Maupas designated as sexual maturity. The body size is somewhat less than at earlier periods, although this is by no means a constant feature, size depending chiefly on the rate of division and so only indirectly on age. The plasticity at this period is remarkable, and the cortical plasm is so sticky that two organisms striking each other will fuse at the point of contact. I have had *Paramecia* in culture during an epidemic of conjugation with the protoplasm so highly miscible that amorphous groups of partially fused *Paramecia* were formed by the accidental union of from three to nine individuals, dozens of such groups whirling round and round amongst the normally conjugating pairs.

In *Tetramitus*, *Cercomonas*, or other similar flagellates with a firm contour and a definite shape, the organisms in the period of adolescence become plastic or even amœboid, and like *Paramecium* they conjugate while in this condition.

Among the morphological changes that occur during this period of adolescence none are more subtle or more difficult to interpret than those of the nucleus; indeed, we are here brought face to face with one of the fundamental problems of modern biology — the maturation phenomena. While these are undoubtedly general biology or cell problems, there is another phenomenon connected with the nucleus of protozoa at this period which, despite the ingenious analogies of Goldschmidt, has no satisfactory simulacrum in metazoa, — the formation of *chromidia*. Using the term *idiochromidium* proposed by Mesnil to designate the distributed chromatin prior to gamete formation, we see in this material a practically characteristic sexual substance which, appearing prior to conjugation, belongs primarily to this period of adolescence. In all those forms in which multiple fragmentation has been described, the fragments become the nuclei of conjugating gametes (examples in *Coccidium schubergi* and *Entamœba* according to Schaudinn). In rhizopods, however, especially in the testacea, this nuclear distribution begins at an earlier period in the life history, and the *idiochromidium* is characteristic of the ordinary vegetative forms (examples in *Diffugia* [Zueltzer], *Centropyxis* [Schaudinn], etc.). This chromatin differentiation and distribution indicates a curious change in the chemical balance of nucleus and cytoplasm during the period of adolescence; a change which may appear earlier or later in different types, the earliest appearance being seen in the case of infusoria where the differentiation into vegetative and germinal nuclei occurs immediately after conjugation. In the majority of cases, however, the appearance of the *idiochromidium* and the various stages leading up to its formation may be taken as evidence of advancing age of the series of individuals and as a token of the near completion of the cycle.

So widespread is this nuclear phenomenon and so well established in protozoa studies that we are justified in some cases in arguing from this phenomenon alone that adolescence and the

period of sexual union is imminent. For example, in the case of the ordinary forms of *Amœba* it is remarkable that sexual processes have never been observed, although the engulfing of one individual by another has been interpreted as a conjugation phenomenon. One series of forms has recently come to my notice which seems to indicate a sexual process. The ordinary form of *A. proteus*, usually has but a single nucleus, but in one culture in my laboratory after a long series of uninucleated forms a culture appeared in which the individuals were in various phases of encystment or in nuclear fragmentation immediately prior to encystment. It was found that the single primary nucleus divides by mitosis; that these divide again and so on until as many as seventy large nuclei may fill the body of the amœba. In some cases before this number is reached the larger nuclei begin to break down into large granules which become distributed throughout the cell appearing exactly like the idiochromidium of *Diffugia*, *Arcella* or *Centropyxis*. All but one of the primary nuclei are finally disposed of in this way, that one remaining unused even in the final encysted stage. In this final stage the cyst is filled with many reproductive bodies, which from analogy with other rhizopods I interpreted as gametes.

Not only is chromidium formation important in determining the phase of development of a given form, but it may also be of the greatest assistance in proving the protozoön nature of questionable structures found in certain diseased tissues. An interesting example has recently come up in connection with the organism of rabies. This organism, under the name of the Negri bodies, has been looked upon with great suspicion by biologists and pathologists alike, and from its general staining reaction and from its ordinary vesiculated appearance it has been more frequently passed by as an artifact or secretion or degeneration product, than considered as an organism. But during the season just passed, Dr. A. W. Williams, of New York, working with a different method from that ordinarily employed, was able to prove that what appear usually as vesicles in these bodies are in reality substances which take a characteristic nucleus stain with the Giemsa method, and she shows that the Negri bodies are amœboid cells with nuclei in different stages of chromidium

formation. These erstwhile questionable bodies must now go into the protozoan literature under the generic name of *Neurocytes* given by Dr. Williams. When the same method comes to be applied to the small-pox organism, I am confident that the last doubter will be convinced that it, too, is a protozoön, and of the rhizopod type.

Still another feature of this second period is the change in form of the conjugating individuals. We have seen that, with increasing maturity, the organisms of a cycle lose their definite shape, become plastic or amœboid in some cases and conjugate while in this condition. Now it is probably due to the same underlying causes that gametes of relatively minute size are formed. In *Polytoma*, for example, size differences are entirely facultative, two normals, two reduced individuals, or one normal and one reduced individual may unite. From such an indifferent condition we find all intermediate stages to fully established obligatory differences in size between conjugating forms and vegetative forms. This might be interpreted as a purely physiological matter depending upon the general chemical and physical balance in the cell, but with it is bound up very often a second phenomenon,—sexual differentiation, which has a deeper significance than the mere change of form at this period of adolescence. The study of protozoa has thrown no light as yet on this problem, which according to recent experimental and cytological findings, especially Wilson's discoveries on the extra sex-determining chromosome in certain insects, would seem to be a matter of inheritance rather than of controllable physiological balance. In many cases what may be called secondary sexual characters in protozoa are evident from the very outset after conjugation, even the first progeny being sexually differentiated as in *Trypanosoma* or *Adelea*, and this certainly can not be traced to advancing age or to changes in chemical relations of nucleus and cytoplasm.

In some cases these differentiations are not established until some change in external conditions brings them out. Klebs, Dangeard, and others have made different types of flagellates conjugate by changing the temperature or increasing the density in the surrounding medium. The same experiment is performed by mosquitoes and other insects on various parasitic

protozoa, as when some blood dwelling parasite is withdrawn from the hot environment of the mammalian body to the colder regions of a mosquito's digestive tract. If such experiments become obligatory, and it is apparently so in many cases, then certainly the most efficient prophylaxis is getting rid of the all-important intermediate host, a preventive measure that has been so signally successful in the Roman Champagna, in Cuba, in Vera Cruz, and in New Orleans.

The happenings within the body of such intermediate hosts are by far the most important to the parasitic protozoa of all their life processes, for the conjugation period with them, as with all free-living protozoa, is the critical period of the life history, and on it depends whether the race shall be given a new vigor and a new lease of life, or shall pass on into the third period of the life cycle characterized by old age or senescence and death.

The third period in the life cycle of protozoa is characterized by the peculiar cytolytic processes that accompany starvation, by loss in size, by vacuolar degeneration in nucleus and cytoplasm, and by final natural death. The symptoms may precede both physiological and germinal death, and many of the so-called involution forms frequently described in parasitic protozoa may be individuals in this third stage of vitality. At this period, conjugation, as pointed out by Maupas, seems to be impossible, the chance of rejuvenescence is cut off, and the race, now comparable to the worn out somatic cells of a metazoön, becomes extinct.

This series of changes from the fertilized cell to the ultimate extinction by natural death is a consecutive series and forms a clean-cut and well-defined life cycle or unit for all forms of protozoa. The vital processes are vegetative in nature and varying phases may be largely accounted for by the conditions of metabolism. In metazoa we can make a clear distinction between the history of the individual and the history of the race, and in protozoa, with the life cycle as the unit, we can make the same distinction. The ordinary phenomena of vegetative life of the cell, metabolism in all its processes, have to do with digestion, excretion, irritability, growth, and multiplication, and are functions pertaining distinctly to the life cycle and may be considered independently of those which have to do with the continuity of the

species or race. Among the latter are the many processes accompanying fertilization and rejuvenescence, and the phenomena here, although not a part of the life cycle any more than sexual processes are a part of the life history of an individual metazoön, are nevertheless dependent on it and can not be omitted in any adequate account of a life cycle, since the succession of cycles, or the race, depends upon them. It is in this field of phenomena that we find the most fascinating aspects of modern protozoa study, for the problems here are general biological problems, and their solution means the illumination of some of the darkest places in biological science. Let me ask your attention for a few minutes, in concluding, to some features of this general subject that have interested me during the past year. They relate to maturation phenomena, to renewal of vitality after conjugation, and to artificial rejuvenescence in *Paramecium*, all functions of the species or race rather than of the unit life cycle.

One of the deepest problems of general biology, heredity, is bound up with the history of the chromatin in the formation of germ cells, and here in protozoa, as the culminating phenomena of the period of adolescence, we find the same type of maturation as in metazoa or the higher plants. At the present time there seems to be no connection whatsoever between the phenomenon of chromidium formation and maturation, while the residual masses of chromatin that are left to degenerate and disappear in so many protozoa prior to fertilization, are more nearly comparable with the residual chromatin of a germinal vesicle in a metazoön than with maturation of the chromosomes. When the history of the idiochromidium is more perfectly established, we may have further evidence to support the identity of the processes in protozoa and metazoa. The division of the chromidium granules in *Amæba proteus*¹ which I have elsewhere described in some detail may be the equivalent of the maturation divisions of the chromosomes in germ cells of the metazoa. There is more definite evidence of this similarity in other forms of protozoa. In *Trypanosoma noctuæ*, for example, maturation processes entirely similar to those of the higher animals have been de-

¹ "Evidence of a Sexual Phase in the Life Cycle of *Amæba proteus*," *Arch. f. Protist.*, Bd. V., 1904.

scribed by Schaudinn. In his preliminary paper this brilliant observer does not go into the details of the process but states categorically that the male nucleus contains only four of the typical eight somatic chromosomes, while the female nucleus contains a similar four in the shape of tetrads formed by the transverse division into four parts of a longitudinally split spireme thread, which are reduced to four single chromosomes by two successive divisions, one a reducing the other an equational division. Cytologists everywhere are waiting for a confirmation and for a more definite description of this remarkable process which thus resembles very closely the maturation processes of certain metazoa. Other instances of reduction in protozoa have been given from time to time, and are quite sufficient to show that maturation phenomena are as widespread in protozoa as in other forms of life and that their underlying significance is as wide as the entire field of biology. The infusoria perhaps more than other forms of protozoa are generally cited in this connection. Here Maupas early showed that of the divisions of the micronucleus of *Paramecium caudatum* one persists to form the functional male and female pronuclei, while the other three atrophy and disappear in the cytoplasm. Schaudinn showed that in Heliozoa one daughter-nucleus which he compared with a polar body is thrown off to disintegrate and disappear in conjugating Actinophrys, while Hertwig showed that two such bodies are cast off by conjugating individuals of Actinosphærium.

In none of these cases has the finer details of chromosome formation been sufficiently described, and the number of chromosomes has rarely been counted or the actual reduction made out. Hertwig somewhat doubtfully claimed that the number in *Paramecium* is reduced from eight or nine to four or six, but there certainly must have been a mistake in the interpretation of what constitutes the chromosome in this case, for the actual number is many times greater than what he gives. One of the graduate students at Columbia, Miss Cull, has worked with me the past year on the formation of the chromosomes during the conjugation period of *P. caudatum*, and although our results on the maturation divisions are not yet ready for publication, we have proved that the history of the chromatin in the early period

of the maturation process agrees with a remarkable exactness with what occurs in many germ cells. The curious and enigmatic crescent which the micronucleus forms during the early phases of maturation, is the form assumed by the nucleus during the stages of synapsis and contraction, and the first spindle develops from this crescentic nucleus with its longitudinally divided chromosomes in the form of heterotypical loops. The condensed chromatin of the resting nucleus is first broken up into many fine granules which become arranged in lines radiating backwards from the intranuclear division center. The nucleus elongates in the direction of these lines until it is seven or eight times the original length. Then, with the growth of the division center, the entire structure becomes crescent shaped and many times the volume of the original micronucleus. The long lines of chromatin appear to form a confused network, but in the contraction phase which follows shortly after the crescent, it can be seen that these lines of chromatin are much thicker than they were and distinctly double, and although not conclusively demonstrated, the most reasonable interpretation regarding their origin is that the long lines of chromatin unite side by side in a typical parasynapsis.

It has been customary to describe the pronuclei in *Paramecium* as fusing while in the spindle form. In a general way this is true, but it is only an elongated form assumed by the nucleus at the time of this union, for the spindle at this time is in no sense a mitotic spindle, the chromatin being in a finely divided state and distributed throughout the nuclei.

It has been generally believed that conjugation brings about a renewal of vitality, a *Verjüngung* or rejeunissement according to Bütschli and Maupas, or an *Erfrischung*, to use a term suggested by Weismann. This interpretation seems to be so obvious on *a priori* grounds that experiments to prove it would appear hardly necessary. In protozoa it is not rejuvenescence strictly speaking but the formation of a new individual, and so also is it in metazoa. It would seem to be easy enough to prove that conjugation actually starts a new race from weakened individuals, but singularly few experiments have been undertaken with this object in view. Some that have been carried out by Miss Cull

and myself during the last year, while proving that conjugation does bring about renewed vigor, also show that the interpretation must be trimmed of some of its generalizations.

It has also been generally assumed in cases of conjugation where, as in *Paramecium*, both individuals are similar in size, and where conjugation is only temporary, that both individuals are fertilized, but according to these experiments which I have cited Miss Cull has shown that, in the majority of cases, while one individual of the original pair is markedly vigorous after conjugation, the other one either forms a weak strain or dies off at an early period.

It would seem from these results that in cases like this of isogamous conjugation we can catch a glimpse at least of the same principle that operates in the fertilization of an egg by a spermatozoön, where one cell loses its identity and continues to exist only in conjunction with another cell. It appears to be a case of incipient fertilization and indicates some physiological difference between the conjugating individuals analogous to that between spermatozoön and egg.

The analysis of the conditions governing conjugation has not yet been carried very far. My own experiments show that Maupas's three conditions can not hold. Hunger apparently has nothing to do with it, and diverse ancestry is not essential, for I have obtained as large a percentage of successful endogamous as exogamous pairings and have carried one endogamous exconjugant through 379 generations. Maturity, however, the third "condition" postulated by Maupas, seems to be necessary, understanding by this term the peculiar state of cytoplasm and nucleus when conjugation is possible and a condition which can be induced by artificial means such as change in temperature or of density in the surrounding medium.

There is yet another matter that I wish to speak of in connection with conjugation and rejuvenescence, and that is the question of artificial rejuvenescence, a matter which has an important bearing, it seems to me, in all protozoan life histories. In a series of experiments which I carried on for twenty-three months with one race of *Paramecium*, it was found that periodic reductions of vitality occurred at intervals of about six months. At such

periods of "depression" the race under cultivation would have died out entirely, had not stimuli in the form of extracts of different substances (beef, pancreas, brain, etc.) been applied. With the aid of such restoratives on three different occasions, the race was carried through four "cycles" of activity and through 742 generations.

There is no doubt that the organisms would have died of a real physiological exhaustion, had they not been artificially stimulated, and even when the race finally ran out, it was not from physiological exhaustion in the same sense, for stimuli had again been successful in restoring the vegetative functions. It was due to some more deeply lying trouble, and the results confirmed Hertwig's view of "germinal" death as contrasted with "physiological" death, and, as I have elsewhere pointed out, they demonstrate that in the protozoön as in the metazoön we may distinguish between somatic and germinal protoplasm.

We can readily understand how such periods of depression may be overcome in nature and stimulation effected by changes in the immediate environment or, in parasitic forms, by changes in the blood, and continued activity of certain parasites or appearance of the recidive in malaria, etc., may thus be accounted for. But the organisms themselves have an efficient means of bringing about this renewal of vitality. It seems probable that some original supply of physiological energy is continually drawn upon by the vegetative organisms, some "potential of vitality," which, like the charge of a battery, may become exhausted. As in a battery this potential can be renewed by artificial means, but unlike a battery it can also charge itself by the process of parthenogenesis. This has been shown by Schaudinn to take place in the malaria organism, *Plasmodium vivax* and in *Trypanosoma noctuæ* of the owl. Here, as in some insects, rejuvenescence is brought about by the union of the kinetonucleus and the vegetative nucleus, and is quite analogous to the fertilization in some metazoa of the egg nucleus by a polar body nucleus. Protozoan organisms which are thus restored by parthenogenesis seem to carry with them at least one extra charge of vitality, and we have no basis for speculating as to the length of time that such parthenogenetic processes may continue in a race. In the experi-

mental work on *Paramecium* artificial parthenogenesis was successful at three different periods of depression but failed on the fourth, and we may infer that normal parthenogenesis has only a limited success and that sooner or later the race must come to an end unless conjugation and nuclear reorganization take place. It is conceivable that parthenogenesis is only a means of offsetting physiological death or in other words, of stimulating physiological activities in protoplasm in which the potential of development is not yet exhausted.